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(21) International Application Number: PCT/US99/19721 (22) International Filing Date: 30 August 1999 (30.08.99) (30) Priority Data: 60/098,473 31 August 1998 (31.08.98) US (71) Applicant (for all designated States except US): PROTEOTECH, INC. [US/US]; 12026 - 115th Avenue N.E., Kirkland, WA 98034 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CASTILLO, Gerardo [CA/US]; 11725 - 82nd Avenue S., Seattle, WA 98178 (US). SNOW, Alan, D. [CA/US]; 3812 - 167th Place S.W., Lynnwood, WA 98037 (US). (74) Agent: DWYER, Patrick, M.; Patrick M. Dwyer PC, 1919 One Union Square, 600 University Street, Seattle, WA 98101 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: BLENDED COMPOSITIONS FOR TREATMENT OF ALZHEIMER'S DISEASE AND OTHER AMYLOIDOSES (57) Abstract A pharmaceutical agent for treating an amyloid disease in a patient, wherein the pharmacological agent comprises a therapeutically effective amount of plant matter from a plant of the genus <i>Uncaria</i> , species <i>tomentosa</i> , in combination with a therapeutically effective amount of one or more of the substances from the group of substances consisting of <i>Ginkgo Biloba</i> , Ginseng, Gotu Kola, Echinacea, Vitamin E, Selenium, Niacin or nicotinate, Folic acid, Vitamin B12, and Choline, or from the group of substances consisting of Bilberry, Dong Quai, Aloe Vera, Chromium Polynicotinate, Selenium, Vitamin B12 or cobalamin, Folic acid, Biotin, and Thiamine HCl, or vitamin B1.		

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Title: BLENDED COMPOSITIONS FOR TREATMENT
 OF ALZHEIMER'S DISEASE AND OTHER AMYLOIDOSES

TECHNICAL FIELD

5 The invention relates to blended compositions for treating Alzheimer's Disease and other amyloidoses; more particularly, it relates to blended compositions for therapeutic intervention in Alzheimer's disease and other amyloidoses.

BACKGROUND OF THE INVENTION

Brain Amyloid Prevention and Memory / Recall Optimization

10 It is known that amyloid accumulates in the brains of people as they age. This amyloid is most commonly and most deleteriously in the form of what are known as amyloid plaques. In addition there are amyloid deposits in cerebral blood vessels. These accumulations form a brain amyloid burden that increases with age, so that age is a risk factor for Alzheimer's disease and other amyloidoses.

15 One of the most notable effects of increasing brain amyloid burden, and especially in Alzheimer's Disease, is the gradual deterioration of short term memory; that is, the ability to recall immediately those memories only recently stored.

 Alzheimer's disease in general is characterized by the accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein or A β , in a fibrillar form, existing
20 as extracellular amyloid plaques and as amyloid within the walls of cerebral blood vessels. Fibrillar A β amyloid deposition in Alzheimer's disease is believed to be detrimental to the patient and eventually leads to toxicity and neuronal cell death,

characteristic hallmarks of Alzheimer's disease. Accumulating evidence implicates amyloid as a major causative factor of Alzheimer's disease pathogenesis.

5 A variety of other human diseases also demonstrate amyloid deposition and usually involve systemic organs (i.e. organs or tissues lying outside the central nervous system), with the amyloid accumulation leading to organ dysfunction or failure. In Alzheimer's disease and "systemic" amyloid diseases, there is currently no cure or effective treatment, and the patient usually dies within 3 to 10 years from disease onset. For additional background in this area, the reader is referred to WIPO International publication number WO98/51302 by the same inventors, the text of
10 which is hereby incorporated by reference as if fully set forth herein.

Additional compounds or agents for therapeutic regimes to arrest or reverse amyloid formation, deposition, accumulation and/or persistence that occurs in Alzheimer's disease and other amyloidoses are still needed.

DISCLOSURE OF THE INVENTION

15 An object of the present invention is to use the inner bark and/or roots from *Uncaria tomentosa* (also referred to as Uña de Gato or Cat's claw) for the treatment of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease, type II diabetes and other amyloidoses, in conjunction with one or more of the additional blended ingredients disclosed below to achieve a beneficial synergistic
20 therapeutic effect. *Uncaria tomentosa* or Cat's claw is also referred to as, but not limited to, Paraguayo, Garabato, Garbato casha, Tambor huasca, Una de gavilan, Hawk's claw, Nail of Cat, and Nail of Cat Schuler.

Another object of the present invention is to provide the use of *Uncaria tomentosa* with its blended compounds (regardless of commercial source and regardless
25 of final form for consumption by humans, i.e. pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, bark bundles

and/or bark powder) for inhibition of amyloid formation, deposition, accumulation, and/or persistence, regardless of its clinical setting.

It is yet another object of the invention to meet any or all of the needs summarized above.

5 These and such other objects of the invention as will become evident from the disclosure below are met by the invention disclosed herein.

Application of the invention to these needs is especially beneficial in that the invention is the only system that effectively provides for use of extracts from the inner bark and root parts of *Uncaria tomentosa*, together with hitherto unknown blended
10 additional compounds, to benefit human patients with Alzheimer's disease and other amyloidoses due to the newly discovered ability of *Uncaria tomentosa* in combination with one or more of these blended ingredients, to effectively inhibit amyloid fibril formation, inhibit amyloid fibril growth, inhibit amyloid - proteoglycan interactions, amyloid - glycosaminoglycan interactions, and cause dissolution and/or disruption of
15 preformed amyloid fibrils.

We have earlier discovered and disclosed a naturally occurring plant product, the inner bark and/or roots from the plant *Uncaria tomentosa*, or Cat's Claw, that we call PTI-00703™, in WIPO International publication number WO98/51302 entitled Composition and Methods for Treating Alzheimer's Disease and other Amyloidoses
20 dated November 19, 1998. As disclosed therein, this plant compound alone has surprising efficacy in disrupting and/or dissolving amyloid deposits and other accumulations, and is believed to be a potent inhibitor of amyloid formation in Alzheimer's Disease, Type II Diabetes, and other amyloidoses. It is now also believed that formulations of PTI-00703 with other plant compounds, herbals, minerals, and/or
25 vitamins as disclosed herein have surprising and hitherto unsuspected supplementary efficacy in treating the various amyloidoses addressed by our earlier disclosure for PTI-00703 by itself.

Alzheimer's disease

PTI-00703 is advantageously blended with one or more of the following ingredients for Alzheimer's disease amyloidosis, and for improved brain cognition, memory / recall optimization and the like.

- 5 1) Gingko Biloba, an herb that enhances memory;
- 2) Ginseng, an herb for promoting well-being and energy;
- 3) Gotu Kola, an herb that increases energy, endurance, memory and mental stamina;
- 4) Echinacea, an herb with potent anti-oxidant activity;
- 10 5) Vitamin E, an anti-oxidant;
- 6) Selenium, an anti-oxidant;
- 7) Niacin, or nicotinate, a B vitamin that helps with cell metabolism and cell energy;
- 8) Folic acid, part of B-complex that helps with cardiovascular function and
15 circulatory health;
- 9) Vitamin B12, or cobalamin, important in cell metabolism and cell energy;
- 10) Choline, precursor to acetylcholine that is important in essential brain neurotransmission.

Optimal formulations of PTI-00703 contain one or more of these ingredients.

- 20 It is expected that increasing PTI-00703 dosage should occur with older people (i.e. different regimes for people age 20-40 years old; 40-60 years old and >60 years old) so as best to accommodate the risk factor described above.

These and other features and advantages of the present invention will become more fully apparent when the following detailed description of the invention is read in
25 conjunction with the accompanying figures.

A preferred pharmacological agent preferably has a therapeutically effective amount of *Uncaria tomentosa* in a dosage in the range of from about 10 to 1,000 mg/kg of body weight of the patient, and more preferably in the range of from about 10 to 100 mg/kg of body weight of the patient.

Preferred pharmaceutical agents have a weight percentage of plant extract in the agent is in the range of from about 70% to about 95%, and may also have a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical agent preferably has an amyloid inhibitory activity or efficacy greater than 50%.

5 The plant matter is preferably comprised of commercially obtained pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, bark bundles and/or bark powder, which contain *Uncaria tomentosa*, extracts
10 or derivatives thereof, and may be taken from commercially available gelatin-coated capsules which contain dried-powder of *Uncaria tomentosa*, extracts or derivatives thereof.

A method is also disclosed for treating an amyloid disease in a patient, comprising the step of administering to the patient a therapeutically effective amount
15 of plant matter from a plant of the genus *Uncaria*, species *tomentosa*, in combination with one or more of the additional blend ingredients disclosed above. The plant matter is preferably administered orally or by aerosol spray or in a parenterally injectable or infusible form.

Examples

20 20-40 Years of Age

400 mg of PTI-00703 plus 50 mg of a mixture of all ten of the above listed ingredients (i.e. 5 mg per ingredient) = 450 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 3X day.

40-60 Years of Age

25 600 mg of PTI-00703 plus 75 mg of a mixture of all ten of the above ingredients (i.e. 7.5 mg per ingredient) = 675 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 3X day.

>60 Years of Age, or diagnosed Alzheimer's Disease

800 mg of PTI-00703 plus 100 mg of a mixture of all ten of the above ingredients (i.e. 10 mg per ingredient) = 900 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 4X day.

5 Pancreatic Amyloid Diabetes Prevention and Beta Cell Optimization

It is also known that amyloid accumulates in the pancreas of 90% of all patients with Type II Diabetes, and that this amyloid accumulation contributes to pancreatic organ dysfunction. For one thing, amyloid is toxic to beta cells which normally produce insulin, and it has been observed that patients with such amyloid accumulation are at
10 risk of becoming insulin dependent (i.e. Type I Diabetes). For further background on pancreatic amyloidosis, the reader is referred to the inventors earlier WIPO International publication number WO98/51302.

PTI-00703 is advantageously blended with one or more of the following ingredients for type II diabetes amyloidosis and beta cell optimization and the like.

- 15 1) Bilberry, an herb with circulation enhancing properties;
- 2) Dong Quai, and herb that helps maintain immune function, blood pressure, and good circulation;
- 3) Aloe Vera, an herb that helps promote healthy lower intestine function;
- 4) Chromium Polynicotinate, an organic complex of chromium and picolinic acid
20 that works to metabolize the body's fat and cholesterol;
- 5) Selenium, an anti-oxidant that neutralizes free radicals that can otherwise damage cells;
- 6) Vitamin B12, or cobalamin, important in cell metabolism and cellular level energy production;
- 25 7) Folic acid, a part of the B-complex that helps with cardiovascular function and circulatory system health;
- 8) Biotin, a part of the B-complex that also promotes circulatory health;
- 9) Thiamine HCl, or vitamin B1, helps maintain blood sugar levels by increasing metabolic efficiency and helps maintain cardiovascular function.

Optimal formulations of PTI-00703 contain one or more of these ingredients. It is expected that increasing PTI-00703 dosage should occur with older people (i.e. different regimes for people age 20-40 years old and >40 years old and for people who have diagnosed type II diabetes) so as best to accommodate the risk factor described above.

Examples

20-40 Years of Age

300 mg of PTI-00703 plus 45 mg of a mixture of all nine of the above ingredients (i.e. 5 mg per ingredient) = 345 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 3X day.

>40 Years of Age

500 mg of PTI-00703 plus 63 mg of a mixture of all nine of the above ingredients (i.e. 7 mg per ingredient) = 563 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 3X day.

Diagnosed Type II Diabetes

700 mg of PTI-00703 plus 90 mg of a mixture of all ten of the above ingredients (i.e. 10 mg per ingredient) = 790 mg total, with a weight ratio of 8:1 PTI-00703 to total other ingredients. Mixture may be taken orally (or the like) in gel caps 4X day.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph of a sequence of a Thioflavin T fluorometry assay of one of the combinations of the invention.

FIGURE 2 is a graph of a Thioflavin T fluorometry assay utilized to assess effectiveness of one of the combinations of the invention for its inhibition of Alzheimer's A β -A β interactions.

FIGURE 3 is a graph of a Thioflavin T fluorometry assay utilized to assess effectiveness of one of the combinations of the invention for its inhibition of Alzheimer's A β -proteoglycan/glycosaminoglycan (PG/GAG) interactions.

FIGURE 4 is a graph of a Thioflavin T fluorometry assay utilized to assess effectiveness of one of the combinations of the invention in dissolution of pre-formed Alzheimer's A β 1-40 amyloid fibrils in a dose-dependent manner.

5 FIGURE 5 is a graph of a Thioflavin T fluorometry assay utilized to assess effectiveness of one of the combinations of the invention in dissolution of pre-formed Alzheimer's A β 1-42 amyloid fibrils in a dose-dependent manner.

BEST MODE OF CARRYING OUT THE INVENTION

Amyloid and Amyloidosis

10 Amyloid is a generic term referring to a group of diverse, but specific extracellular protein deposits which all have common morphological properties, staining characteristics, and x-ray diffraction spectra, further details and information as to which, and as to amyloid as a therapeutic target for Alzheimer's Disease, the reader is referred to the inventors' WIPO International publication number WO98/51302.

15 Uncaria tomentosa

The plant *Uncaria tomentosa*, also known as "Uña de Gato" (in Spanish) or "Cat's claw" (in English) refers to a woody vine which grows within the Peruvian Amazon rain forest. For additional and further information and background on *uncaria tomentosa*, the reader is also referred to the inventors' WIPO International
20 publication number WO98/51302.

Although some health care providers have suggested that *Uncaria tomentosa* may be used to treat a variety of ailments, nowhere has there been any use, or suggestion of use, of this compound for the treatment of amyloid formation, deposition, accumulation and/or persistence, such as that which occurs in the amyloidoses,
25 including Alzheimer's disease, and nowhere is it suggested that certain other compounds might have synergistic or supplemental efficacy in combination with *uncaria tomentosa* in treating amyloidoses. The present invention clearly demonstrates the effectiveness of *Uncaria tomentosa* and its combinations for 1) inhibition of Alzheimer's A β amyloid fibril formation (important for patients in early

to mid-stage Alzheimer's disease), 2) inhibition of Alzheimer's amyloid fibril growth (important for patients in early to mid-stage Alzheimer's disease), 3) inhibition of Alzheimer's amyloid-PG/GAG interactions (important for patients in all stages of Alzheimer's disease) and 4) causing the dissolution/disruption of preformed Alzheimer's disease amyloid fibrils. In addition, the present invention and its combinations is effective in causing the dissolution of islet amyloid fibrils (i.e. amylin) and therefore serves as an effective treatment for ~90% of type II diabetic patients who have islet amyloid accumulation in the pancreas.

Methodologies Employed for *In Vitro* Testing

10 Generation of "Water Extracts" for *In Vitro* Testing

For the procedure to generate water extracts of PTI-00703, 500mg PTI-00703 were extracted with 3ml of distilled water (Baxter) and placed in microcentrifuge tubes. The microcentrifuge tube contents were then vortexed by hand for 3-4 minutes, and then allowed to stand for 1-2 minutes. The samples were then centrifuged on a microcentrifuge (Eppendorf, model 5415V) for 30 minutes at 14,000 Xg (at room temperature). Following centrifugation, the supernatants were collected and designated as the "water extracts" used for testing as described below.

Examples

The following examples are put forth so as to provide those with ordinary skill in the art with the disclosure and description of the identification and use of commercially available *Uncaria tomentosa* and disclosed blend ingredients to inhibit amyloid fibril formation, inhibit amyloid fibril growth, inhibit amyloid-PG/GAG interactions, and cause dissolution/disruption of preformed amyloid fibrils. However, it should not be construed that the invention is limited to these specific examples.

25 The PTI-00703TM tested is in the form of Cat's Claw Bark Powder, and the blend testing illustrated below is of 350mg of Cat's Claw Bark Powder and 40mg of Ginkgo biloba powder extract, or Ginkgo biloba leaf extract containing standardized 24% ginkgoflavoglycosides and 6% terpene lactones; total 390mg per test capsule.

Study 1: Testing to Assess Effects on Alzheimer's Disease Amyloid Fibril Formation

A previously described method of measuring amyloid fibril formation utilizing Thioflavin T fluorometry (H Naiki et al, Lab. Invest. 65:104-110, 1991; H Levine III, Protein Sci. 2:404-410, 1993; H Levine III, Amyloid: Int. J. Exp. Clin. Invest. 2:1-6, 1995; H Naiki and K. Nakakuki, Lab. Invest. 74:374-383, 1996) was employed initially to identify whether PTI-00703 and PTI-00703 blended with Ginkgo biloba were capable of inhibiting Alzheimer's A β 1-40 amyloid fibril formation. Using this sensitive assay, any decreases or increases in fluorescence was previously shown to correlate with a decrease or increase in the amount of amyloid fibrils (H Naiki et al, Lab. Invest. 65:104-110, 1991; H Levine III, Protein Sci. 2:404-410, 1993; H Levine III, Amyloid: Int. J. Exp. Clin. Invest. 2:1-6, 1995; H Naiki and K. Nakakuki, Lab. Invest. 74:374-383, 1996), allowing one to determine the identity and extent of potential inhibitors and/or enhancers of amyloid fibril formation.

In one study, the dose-dependent effects of PTI-00703 and its Ginkgo biloba blend on Alzheimer's A β (1-40) fibril formation was assessed by Thioflavin T fluorometry. Thioflavin T is known to bind to fibrillar amyloid proteins, and an increase in fluorescence correlates with an increase in amyloid fibril formation, whereas a decrease in fluorescence correlates with a decrease in amyloid fibril formation. The Alzheimer's A β protein (1-40) when incubated at 37°C tends to spontaneously form amyloid fibrils which increase in quantity over time. In this study, we tested for ability to inhibit the Alzheimer's amyloid A β protein from forming fibrils over a 1 week period. For these studies, 300 μ l of 25 μ M A β (1-40) (Bachem Inc., Torrance, CA, USA; Lot #T20824) in 150 mM TRIS, 10mM NaCl, pH 7.0 (TBS) was incubated in microcentrifuge tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of increasing concentrations (i.e. 0.01 μ l, 0.1 μ l, 0.5 μ l and 1.0 μ l) of a water extract (described below) of PTI-00703 and PTI-00703 with Ginkgo biloba (obtained as described above).

To assess the dose-dependent effects of these substances on A β (1-40) fibril formation, 50 μ l aliquots were taken from each tube (as described above) for analysis

at 1 hr, 1 day, 3 days, and 1 week. For each determination described above, following each incubation period, 50 μ l of A β +/- increasing concentrations of a water extract were added to 1.2ml of 100 μ M Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50mM NaPO₄ (pH 6.0). Studies indicated that increasing concentrations of fibrillized A β gave a proportional increase in fluorescence in the presence of 100 μ M Thioflavin T, ruling out the presence of any disproportionate inner filter effects in these studies. Fluorescence emission at 482 nm was measured on a Turner instrument-model 450 fluorometer at an excitation wavelength of 450 nm. For each determination, the fluorometer was calibrated by zeroing in the presence of the Thioflavin T reagent alone, and by setting the 50 ng/ml riboflavin (Sigma Chemical Co., St. Louis, Mo) in the Thioflavin T reagent to 1800 fluorescence units. All fluorescence determinations were based on these references and any fluorescence given off by any of the compounds tested in the presence of the Thioflavin T reagent was always subtracted from all pertinent readings.

For all fibrillogenesis studies utilizing Thioflavin T fluorometry, as disclosed herein, comparisons of amyloid protein in the presence or absence of test compounds were based on paired Student's t tests with data shown as mean +/- standard deviation. Significance was reported at the 95% ($p < 0.05$) and 99% ($p < 0.01$) confidence levels.

Study 2: Testing to Assess Effects on Alzheimer's Disease Amyloid Fibril Growth

In Alzheimer's disease and other amyloidoses, amyloid fibril growth is believed to involve amyloid protein self-interactions (i.e. A β -A β interactions). Any potential effective therapeutic agent for amyloid deposition, accumulation and/or persistence should also be capable of causing an inhibition of amyloid protein self-interactions. This is important for preventing any new amyloid fibril formation when treating Alzheimer's disease patients at early stages of the disease. ELISA methodologies (i.e. solid phase binding assays) were therefore used to identify compounds which were capable of inhibiting A β -A β interactions (i.e. Alzheimer's amyloid fibril growth).

A β (1-40) was first labeled with biotin according to the following protocol. 1 mg of A β (1-40) (Bachem Inc., Torrance, CA, USA; Lot #WL934) was dissolved in 200 μ l of PBS (pH 8.0) and incubated for 1 week at 37°C. The fibrillar A β solution was then added to 0.2mg of a biotinylation agent [(sulfosuccinimidyl-6-(biotinamido) hexanoate)](sulfo-NHS LC-Biotin) and incubated for 45 minutes at room temperature (according to the manufacturer's protocol; Pierce). To remove excess sulfo-NHS-LC-Biotin not incorporated into A β , 25 μ l of 3M sodium acetate and 1 ml of ethanol were added to the solution, vortexed and then centrifuged at 14,000 Xg for 20 minutes. The supernatant was then discarded and the pellet was resuspended in 200 μ l of distilled water, and reprecipitated with ethanol containing 2.5% of 3M sodium acetate. The centrifugation steps (described above) were then repeated. The pellet which contained fibrillized A β which was biotinylated (at the non self interacting region of A β) was then resuspended in 1 ml of distilled deionized water. The amount of biotin incorporated was then determined using the HABA (2-(4'-hydroxyazo benzene)benzoic acid) method (according to the manufacturer's protocol; Pierce).

2 μ g of unlabeled A β in 40 μ l of Tris-buffered saline containing 100mM Tris-HCl, 50 mM NaCl, 3 mM NaN₃, pH 7.0 (TBS) was allowed to bind overnight at 4°C to microtiter wells (Nunc plates, Maxisorb). The next day all of the microtiter wells were blocked for 2 hours by incubating with 300 μ l of TBS with 0.05% Tween-20 (TTBS) plus 2% bovine serum albumin (BSA)(obtained from the Sigma Chemical Company, St. Louis, MO, USA). Then, 100 μ l of 12.5 μ M biotinylated A β 1-40 in TTBS, in the presence or absence of 1 μ l of water extracts (described above) were placed in wells (in triplicate) containing substrate bound unlabeled A β or blank, and allowed to bind overnight at 4°C. The next day, the wells were rinsed 3 times with TTBS, and then probed for 2 hours with 100 μ l of streptavidin peroxidase or anti-biotin peroxidase (1:500 dilution of a 2 μ g/ml solution)(Sigma Chemical Co., St. Louis, MO) in TTBS containing 0.1% BSA. The wells were then rinsed 3 times with TTBS and 100 μ l of a substrate solution (OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO) was added to each

well and allowed to develop for 5 minutes or until a significant color change was observed.

The reaction was stopped with 50 μ l of 4N H₂SO₄ and read on a Model 450 microplate reader (Biorad, Hercules, CA, USA) at 490nm.

5 Study 3: Testing to Assess Effects on Alzheimer's Disease A β -Glycosaminoglycan Interactions

One study was implemented to determine whether the test compounds were effective inhibitors of A β -proteoglycan/glycosaminoglycan (PG/GAG) interactions. Since PGs/GAGs have been found to accumulate in amyloid deposits and are believed
10 to prevent the body's natural ability to remove unwanted "amyloid" (reviewed in Snow and Wight, Neurobiology Aging 10:481-497, 1989), an inhibitor of A β -PG/GAG interactions is a desirable additional target for an amyloid therapeutic. In this study a solid phase binding immunoassay was utilized to determine whether the test compounds were effective inhibitors of A β -PG/GAG interactions.

15 12 μ g of perlecan glycosaminoglycans (isolated from the Engelbreth-Holm-Swarm sarcoma as previously described (Castillo et al, J. Neurochemistry 69:2452-2465, 1997) in 40 μ l of Tris-buffered saline containing 100 mM Tris-HCl, 50 mM NaCl, 3 mM NaN₃, pH 7.0 (TBS) was allowed to bind overnight at 4°C to microtiter wells (Nunc plates, Maxisorb). The next day all of the microtiter wells
20 were blocked for 2 hours by incubating with 300 μ l of TBS with 0.05% Tween-20 (TTBS) plus 1% bovine serum albumin (BSA). 100 μ l of A β 1-40 (12.5 μ M) (Bachem Inc., Torrance, CA, USA; Lot ##T20824) in TTBS containing 1% albumin in the presence or absence of 1 μ l of a water extract of the test compound, PTI-00703 + Ginkgo biloba, were placed in wells (in triplicate) containing substrate bound perlecan GAGs or blank,
25 and allowed to bind overnight at 4°C. The next day, the wells were rinsed 3 times with TTBS, and then probed for 2 hours with 100 μ l of biotinylated anti-4G8 and anti-6E10 (Senetek, Maryland Heights, Missouri) diluted 1:2000 with TTBS. Bound antibodies were then probed with 100 μ l of streptavidin-peroxidase or anti-biotin peroxidase (1:500 dilution of a 2 μ g/ml solution; Sigma Chemical Co., St. Louis, MO) in TTBS for 1 hour.
30 The wells were then rinsed 3 times with TTBS and 100 μ l of a substrate solution

(OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO) was added to each well and allowed to develop for 5 minutes or until a significant color change was observed. The reaction was stopped with 50 μ l of 4N H₂SO₄ and read on a Model 450 microplate reader (Biorad, Hercules, CA, USA) at 490nm.

5 Study 4: Testing to Assess Dose-Dependent Effects on Causing a Dissolution/Disruption of Pre-Formed Alzheimer's Disease Amyloid 1-40 Fibrils

One study was implemented to determine whether extracts of the test compounds were capable of causing a "dissolution" or "disruption" of pre-formed Alzheimer's disease amyloid fibrils. This type of activity would be important for any
10 potential anti-amyloid drug which can be used in patients who already have substantial amyloid deposition in organs and/or tissues. For example, Alzheimer's disease patients in mid-to late stage disease have abundant amyloid deposits in their brains as part of both neuritic plaques and cerebrovascular amyloid deposits. A natural therapeutic agent capable of causing dissolution of pre-existing amyloid would be
15 advantageous for use in these patients who are at latter stages of the disease process.

For this study, 1 mg of A β (1-40)(Bachem Inc., Torrance, CA, USA; Lot #T20824) was dissolved in 1.0 ml of double distilled water (1mg/ml solution) and then incubated at 37°C for 1 week to cause abundant Alzheimer's amyloid fibril formation. 25 μ M of fibrillized A β was then incubated in triplicate for 2 hours at 37°C in a total
20 final volume of 60 μ l TBS, in the absence or presence of increasing concentrations (i.e. 0.01 μ l, 0.1 μ l, 0.5 μ l, and 1.0 μ l) of test compound water extracts. Following a 2 hour incubation, 50 μ l aliquots were added to 1.2ml of 100 μ M Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50mM NaPO₄ (pH 6.0) for fluorometry readings as described in experiment 1 described above.

25 Study 5: Testing to Assess Dose-Dependent Effects on Causing a Dissolution/Disruption of Pre-Formed Alzheimer's Disease Amyloid 1-42 Fibrils

The amyloid fibrils of Alzheimer's disease primarily consist of A β in a form containing residues 1-40 or 1-42. The longer variant of A β contains two hydrophobic residues which cause substantial fibril formation almost immediately (Castillo et al,
30 J. Neurochem. 69:2452-2465, 1997). A β 1-42 is also believed to be the predominant

form of A β existing in Alzheimer's amyloid plaques, whereas A β 1-40 is believed to be the predominant form of A β existing in Alzheimer's cerebrovascular amyloid deposits (Tamaoka et al, Br. Res. 679:151-156, 1995; Biochem. Biophys. Res. Comm. 205:834-842, 1994). The next study was therefore implemented to determine whether the test compound also causes dissolution/disruption of pre-formed A β (1-42) amyloid fibrils and whether this effect was long-lasting.

For this study, the method of Thioflavin T fluorometry as described in Experiment 1 was used. Briefly, 60 μ l of 25 μ M of A β (1-42) (Bachem Inc, Torrance, CA, USA; Lot # 516817) in TBS (pH 7.0) either alone, or containing increasing amounts (i.e. 0.01 μ l, 0.1 μ l, 0.5 μ l, and 1.0 μ l) of test compound water extracts were incubated in microcentrifuge tubes at 37°C for 48 hours (in triplicate).

Results

Study 1: Dose-Dependent Inhibition of Alzheimer's Disease Amyloid Fibril Formation

As shown in Figure 1, the effects of various amounts (i.e. 0.01 μ l, 0.1 μ l, 0.5 μ l and 1.0 μ l) of PTI-00703 and Ginkgo biloba test compound on Alzheimer's A β (1-40) amyloid fibril formation was evaluated over a 1-week incubation period. Following a freeze-drying experiment to determine the weight of each of the water extracts (at each of the dilutions used), the following data for test compound water extracts was generated:

1 μ l of water extract = 23.0 μ g of compound; 0.5 μ l of water extract = 11.5 μ g; 0.1 μ l of water extract = 2.3 μ g; 0.01 μ l of water extract = 0.23 μ g.

In this study, freshly suspended A β (1-40) alone, following a 1-hour incubation at 37°C, demonstrated an initial fluorescence of 142 \pm 53 fluorescence units. During the 1-week incubation period, there was a gradual increase in the fluorescence of A β (1-40) alone, increasing 3.4-fold from 1 hour to 3 days, with a peak fluorescence of 487 \pm 82 fluorescence units observed at 3 days (Figure 1). A significant inhibition ($p < 0.05$) of A β 1-40 amyloid fibril formation by 1.0 μ l of test compound was detected as early as 1 hour of incubation. Significant dose-dependent inhibition by increasing concentrations of the test compound on A β 1-40 amyloid fibril formation was observed.

at all time points including 1 hour, 1 day, 3 days and 1 week. At 1 hour, 0.5 μ l (i.e. 11.5 μ g) and 1.0 μ l (i.e. 23.0 μ g) of water extract inhibited A β 1-40 amyloid fibril formation by 68% and 77%, respectively. At 1 day, 0.5 μ l (i.e. 11.5 μ g) and 1.0 μ l (i.e. 23.0 μ g) of a water extract inhibited A β 1-40 amyloid fibril formation by 62% and 79%, respectively. At 1 week, increasing concentrations of test compound inhibited A β 1-40 fibril formation in a dose-dependent manner, such that 0.1 μ l (i.e. 2.3 μ g), 0.5 μ l (i.e. 11.5 μ g) and 1.0 μ l (i.e. 23.0 μ g) of water extract inhibited A β 1-40 amyloid fibril formation by 39%, 76%, and 86%, respectively. This initial data indicated that the test compound, PTI-00703 + Gingko biloba, was a potent inhibitor of Alzheimer's amyloid fibril formation and exerted its effects in a dose-dependent manner.

Study 2: Potent Inhibitor of Alzheimer's Disease Amyloid Fibril Growth

As shown in Figure 2, test compound water extract was extremely effective in causing a significant reduction in A β -A β interactions. The extract caused a significant ($p < 0.01$) 82% inhibition of A β -A β interactions. This data demonstrated that the test compound was a potent inhibitor of A β -A β interactions, indicative of inhibition of amyloid fibril growth.

Study 3: Inhibition of Alzheimer's Beta-Amyloid Protein-Glycosaminoglycan Interactions

As shown in Figure 3, the test compound significantly ($p < 0.01$) inhibited A β -perlecan GAG interactions by 54%. This data demonstrated that it was also an inhibitor of beta-amyloid protein-PG/GAG interactions.

Study 4: Dose-Dependent Disruption of Pre-Formed Alzheimer's A β (1-40) Amyloid Fibrils

As shown in Figure 4, water extracts of the test compound caused a dose-dependent dissolution/disruption of pre-formed A β 1-40 fibrils within a 2-hour incubation period. For example, 0.5 μ l (i.e. 11.5 μ g) and 1.0 μ l (i.e. 23.0 μ g) of water extract caused a significant ($p < 0.01$) 68% and 89% dissolution/disruption of A β 1-40 amyloid fibrils, respectively. On the other hand, 0.1 μ l (i.e. 2.3 μ g) of water extract still caused a significant ($p < 0.01$) 51% dissolution/disruption of A β 1-40 amyloid fibrils, whereas 0.01 μ l (i.e. 0.23 μ g) of water extract did not cause a significant dissolution/

disruption of pre-formed A β 1-40 amyloid fibrils. These data demonstrated that the test compound causes a disruption/dissolution of pre-formed Alzheimer's disease A β 1-40 amyloid fibrils in a dose-dependent manner. Confirmation of the "dissolution effect" of the test compound, PTI-00703 + Ginkgo biloba, on Alzheimer's disease A β 1-40 fibrils was demonstrated by Congo red staining assays, whereby a reduction of congophilia (i.e. red/green birefringence when viewed under polarized light, and which represents a dissolution/disruption of the amyloid fibrillar structure) was observed when A β amyloid fibrils were treated for 2 hours (not shown).

Study 5: Dose-Dependent Disruption of Pre-Formed Alzheimer's A β (1-42) Amyloid Fibrils

As shown in Figure 5, the water extracts also caused a dose-dependent dissolution/disruption of pre-formed A β 1-42 fibrils within a 2-hour incubation period. For example, 0.5 μ l (i.e. 11.5 μ g) and 1.0 μ l (i.e. 23.0 μ g) of water extract caused a significant ($p < 0.01$) 28% and 64% dissolution/disruption of A β 1-40 amyloid fibrils, respectively. On the other hand, 0.1 μ l (i.e. 2.3 μ g) of water extract only caused a 25% dissolution/disruption of A β 1-42 amyloid fibrils, whereas 0.01 μ l (i.e. 0.23 μ g) of water extract did not cause a significant dissolution/ disruption of pre-formed A β 1-42 amyloid fibrils. These data demonstrated that the test compound also causes a disruption/dissolution of pre-formed Alzheimer's disease A β 1-42 amyloid fibrils in a dose-dependent manner.

Comparisons of Data

Comparisons were made to equal volumes (not weights) of water extracts tested; it is important to note that PTI-00703 and PTI-00703 with Ginkgo biloba were tested at equal volumes of water extracts, and nearly equal weights. Tests results of PTI-00703 alone are not detailed here, having been earlier reported.

1) Inhibition of Alzheimer's Amyloid Fibril Formation

This measure of inhibition of amyloid fibril formation is important as an assessment of the compounds potential as a preventative for normal aging and for early stages of Alzheimer's disease.

A) Inhibition at 1 hour (0.5 μ l of a water extract): PTI-00703 - 86% inhibition; PTI-00703 with Ginkgo biloba - 68% inhibition.

B) Inhibition at 1 week (0.5 μ l of a water extract): PTI-00703 - 80% inhibition; PTI-00703 with Ginkgo biloba - 76% inhibition.

5 2) Inhibition of Alzheimer's Amyloid Fibril Growth

This measure of inhibition of amyloid fibril growth is important as an assessment of the compound's potential as a preventative for normal aging and for early to mid-stages of Alzheimer's disease.

PTI-00703 - 71% inhibition; PTI-00703 with Ginkgo biloba - 82% inhibition.

10 3) Inhibition of Alzheimer's Beta-Amyloid Protein-Glycosaminoglycan Interactions

This measure of inhibition of beta-amyloid protein-glycosaminoglycan interactions is important as an assessment of the compound's potential to inhibit tissue deposition of amyloid, important for normal aging and for all stages of Alzheimer's disease.

15 PTI-00703 - 97% inhibition; PTI-00703 with Ginkgo biloba - 54% inhibition.

4) Dissolution/Disruption of Alzheimer's Disease A β 1-40 Fibrils

This measure of dissolution/disruption of pre-formed A β 1-40 fibrils is important as an assessment of the compound's potential for later stages of aging and for mid-to-late stages of Alzheimer's disease.

20 A) Dissolution with 0.5 μ l of a water extract): PTI-00703 - 63% dissolution; PTI-00703 with Ginkgo biloba - 68% dissolution.

B) Dissolution with 1.0 μ l of a water extract): PTI-00703 - 83% dissolution; PTI-00703 with Ginkgo biloba - 89% dissolution.

5) Dissolution/Disruption of Alzheimer's Disease A β 1-42 Fibrils

25 This measure of dissolution/disruption of pre-formed A β 1-42 fibrils is important as an assessment of the compound's potential use for later stages of aging and for mid-to-late stages of Alzheimer's disease.

A) Dissolution with 0.5 μ l of a water extract): PTI-00703 - 44% dissolution; PTI-00703 with Ginkgo biloba - 28% dissolution.

B) Dissolution with 1.0μl of a water extract): PTI-00703 - 82% dissolution; PTI-00703 with Ginkgo biloba - 64% dissolution.

Synergistic Effects Observed in the Test Formulations

5 The combination of PTI-00703 and Ginkgo biloba in the test formulation appears to lead to certain synergistic effects. For example, it appears to be a better inhibitor of amyloid fibril growth (i.e. Aβ-Aβ interactions) than PTI-00703 alone, and a better agent for causing dissolution of pre-formed Alzheimer's disease amyloid fibrils (both Aβ 1-40 and Aβ 1-42)(although statistically these groups may not be different).

10 Surprisingly, these studies indicate that Ginkgo biloba alone has no real effects on amyloid fibril growth, or dissolution of pre-formed Aβ 1-40 or 1-42 amyloid fibrils (not shown). This observation indicates that there are likely true synergistic effects by the combination of PTI-00703 and ginkgo biloba.

Further Aspects and Utilizations of the Invention

Therapeutic Applications

15 One embodiment of the present invention is to formulate prior to administration in a patient, a pharmaceutical blend comprising Uncaria tomentosa in one or more pharmaceutical acceptable carriers, diluents or excipients.

20 In another preferred embodiment Uncaria tomentosa obtained commercially in any form could be further modulated using suitable carriers, excipients and diluents including lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and
25 suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed response of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 to about 10,000 mg of Uncaria tomentosa (or its active ingredients), more

usually about 500 to about 2,000 mg of *Uncaria tomentosa* (or its active ingredients). However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the clinical condition to be treated, the organ or tissues affected or suspected to be affected
5 with amyloid accumulation, and the chosen route of administration. Therefore, the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in
10 association with a suitable pharmaceutical carrier.

INDUSTRIAL APPLICABILITY

Use of extracts from the inner bark and root parts of *Uncaria tomentosa*, and its blends benefit human patients with Alzheimer's disease and other amyloidoses due to the newly discovered ability of *Uncaria tomentosa* in combination with the disclosed
15 blended ingredients to inhibit amyloid fibril formation, inhibit amyloid fibril growth, inhibit amyloid-proteoglycan interactions, inhibit amyloid-glycosaminoglycan interactions, and cause dissolution and/or disruption of preformed amyloid fibrils.

In compliance with the statute, the invention has been described in language more or less specific as to structural features. It is to be understood, however, that the
20 invention is not limited to the specific features shown, since the means and construction shown comprise preferred forms of putting the invention into effect. The invention is, therefore, claimed in any of its forms or modifications within the legitimate and valid scope of the appended claims, appropriately interpreted in accordance with the doctrine of equivalents.

CLAIMS

We claim:

1. A pharmaceutical agent for treating an amyloid disease in a patient, wherein the pharmacological agent comprises a therapeutically effective amount of plant matter
5 from a plant of the genus *Uncaria*, species *tomentosa*, in combination with a therapeutically effective amount of one or more of the substances from the group of substances consisting of *Ginkgo Biloba*, *Ginseng*, *Gotu Kola*, *Echinacea*, *Vitamin E*, *Selenium*, *Niacin* or *nicotinate*, *Folic acid*, *Vitamin B12*, and *Choline*.
2. The pharmacological agent of claim 1 wherein the therapeutically effective
10 amount of *Uncaria tomentosa* comprises a dosage in the range of from about 10 to 1,000 mg/kg of body weight of the patient.
3. The pharmacological agent of claim 2 wherein the therapeutically effective amount of *Uncaria tomentosa* comprises a dosage in the range of from about 10 to 100 mg/kg of body weight of the patient.
- 15 4. The pharmacological agent of claim 1 wherein said amyloid disease for treatment is selected from the group consisting of the amyloid associated with *Alzheimer's disease*, *Down's syndrome* and *hereditary cerebral hemorrhage with amyloidosis of the Dutch type* (wherein the specific amyloid is referred to as *beta-amyloid protein* or *A β*), the amyloid associated with *chronic inflammation*, various
20 forms of malignancy and *Familial Mediterranean Fever* (wherein the specific amyloid is referred to as *AA amyloid* or *inflammation-associated amyloidosis*), the amyloid associated with *multiple myeloma* and other *B-cell dyscrasias* (wherein the specific amyloid is referred to as *AL amyloid*), the amyloid associated with *type II diabetes* (wherein the specific amyloid is referred to as *amylin* or *islet amyloid*), the amyloid
25 associated with the prion diseases including *Creutzfeldt-Jakob disease*, *Gerstmann-Straussler syndrome*, *kuru* and *animal scrapie* (wherein the specific amyloid is referred to as *PrP amyloid*), the amyloid associated with *long-term hemodialysis* and *carpal tunnel syndrome* (wherein the specific amyloid is referred to as *beta₂-microglobulin amyloid*), the amyloid associated with *senile cardiac amyloid*.

and Familial Amyloidotic Polyneuropathy (wherein the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (wherein the specific amyloid is referred to as variants of procalcitonin).

- 5 5. The pharmacological agent of claim 4 wherein said amyloid disease for treatment is Alzheimer's Disease.
6. The pharmaceutical agent of claim 1 further comprising a pharmaceutically acceptable carrier, diluent or excipient.
7. The pharmaceutical agent of claim 1 wherein the therapeutically effective
10 amount of plant matter has an amyloid inhibitory activity or efficacy greater than 50%.
8. A method of treating an amyloid disease in a patient, comprising the step of administering to the patient a therapeutically effective amount of plant matter from a plant of the genus *Uncaria*, species *tomentosa*, in combination with a therapeutically effective amount of one or more of the substances from the group of substances
15 consisting of *Ginkgo Biloba*, *Ginseng*, *Gotu Kola*, *Echinacea*, *Vitamin E*, *Selenium*, *Niacin* or *nicotinate*, *Folic acid*, *Vitamin B12* or *cobalamin*, and *Choline*.
9. The method claim 8 wherein the therapeutically effective amount of *Uncaria tomentosa* is administered orally.
10. The method claim 8 wherein the therapeutically effective amount of *Uncaria tomentosa* is administered by aerosol spray.
20
11. The method claim 8 wherein the therapeutically effective amount of *Uncaria tomentosa* is administered in a parenterally injectable or infusible form.
12. A pharmaceutical agent for treating an amyloid disease in a patient, wherein the pharmacological agent comprises a therapeutically effective amount of plant matter
25 from a plant of the genus *Uncaria*, species *tomentosa*, in combination with a therapeutically effective amount of one or more of the substances from the group of substances consisting of *Bilberry*, *Dong Quai*, *Aloe Vera*, *Chromium Polynicotinate*, *Selenium*, *Vitamin B12* or *cobalamin*, *Folic acid*, *Biotin*, and *Thiamine HCl*, or *vitamin B1*.

13. The pharmacological agent of claim 12 wherein the therapeutically effective amount of *Uncaria tomentosa* comprises a dosage in the range of from about 10 to 100 mg/kg of body weight of the patient.
14. The pharmacological agent of claim 12 wherein said amyloid disease for
5 treatment is Diabetes.
15. The pharmaceutical agent of claim 12 wherein the therapeutically effective amount of plant matter has an amyloid inhibitory activity or efficacy greater than 50%.
16. A method of treating an amyloid disease in a patient, comprising the step of
10 administering to the patient a therapeutically effective amount of plant matter from a plant of the genus *Uncaria*, species *tomentosa*, in combination with a therapeutically effective amount of one or more of the substances from the group of substances consisting of Bilberry, Dong Quai, Aloe Vera, Chromium Polynicotinate, Selenium, Vitamin B12 or cobalamin, Folic acid, Biotin, and Thiamine HCl, or vitamin B1.

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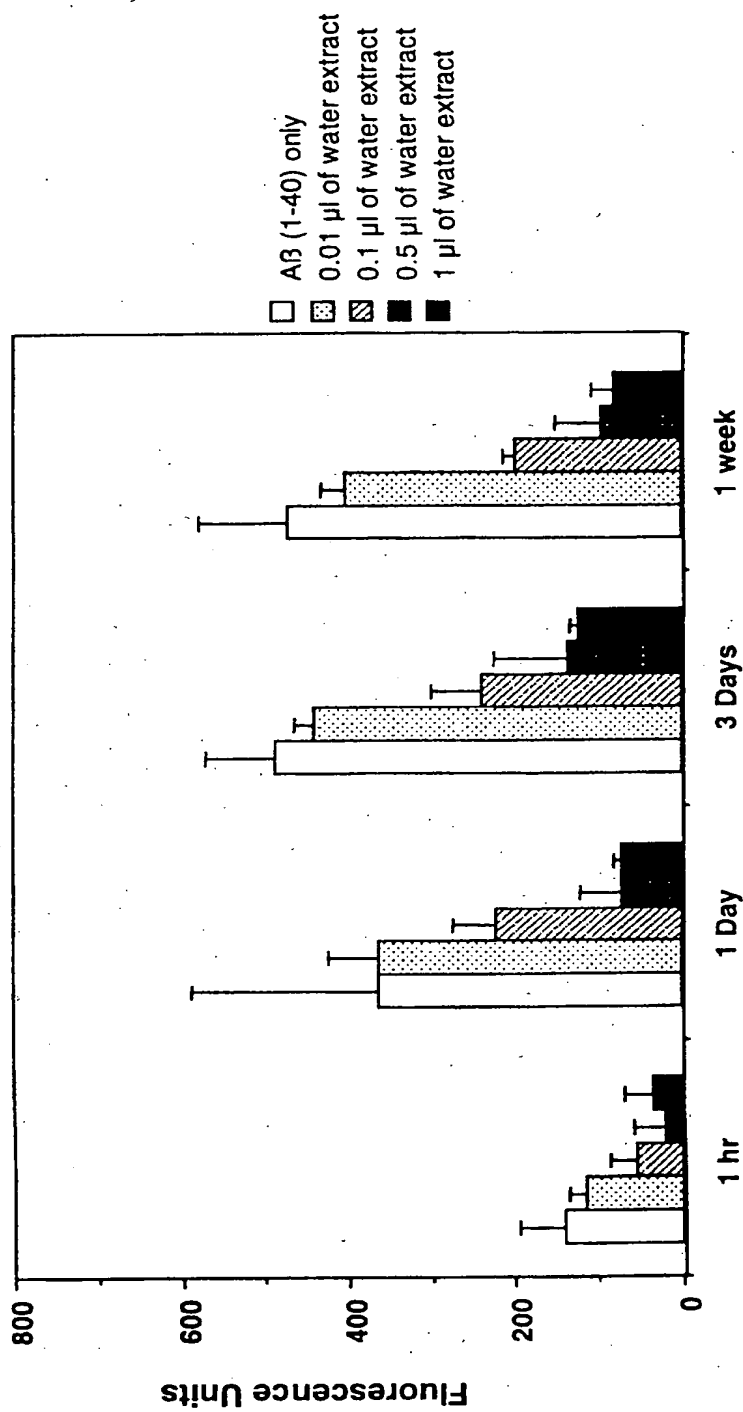


Fig. 1

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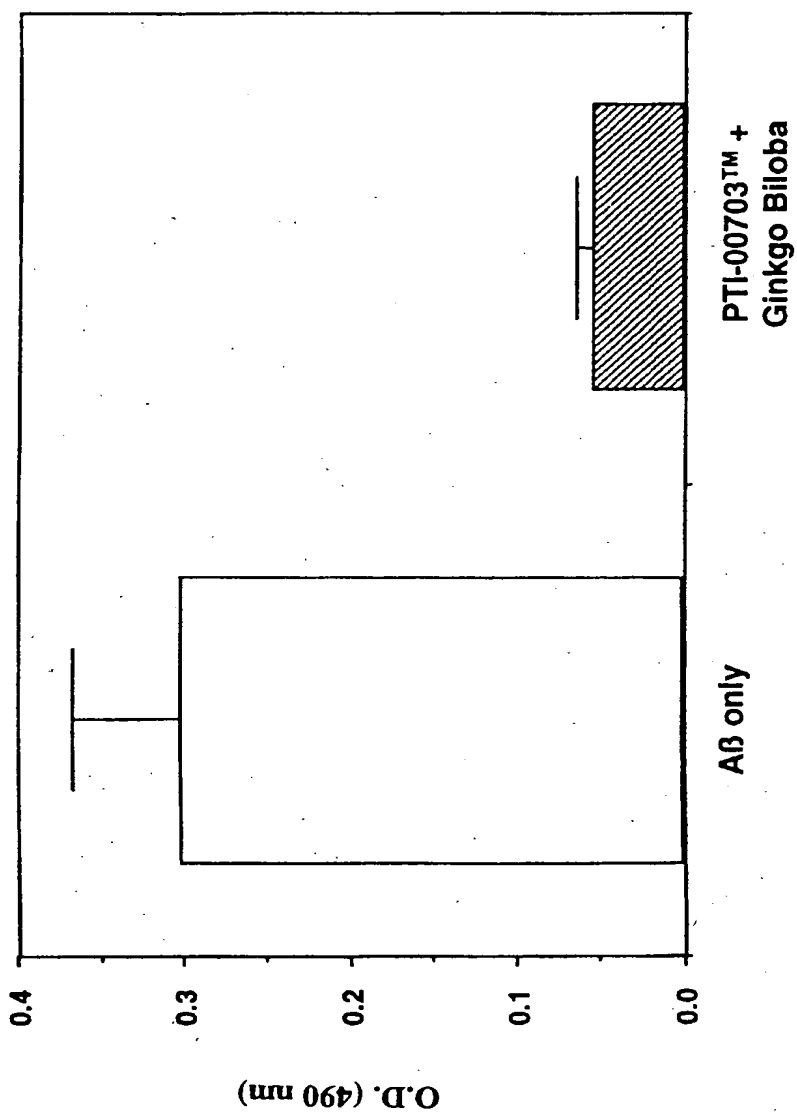


Fig. 2

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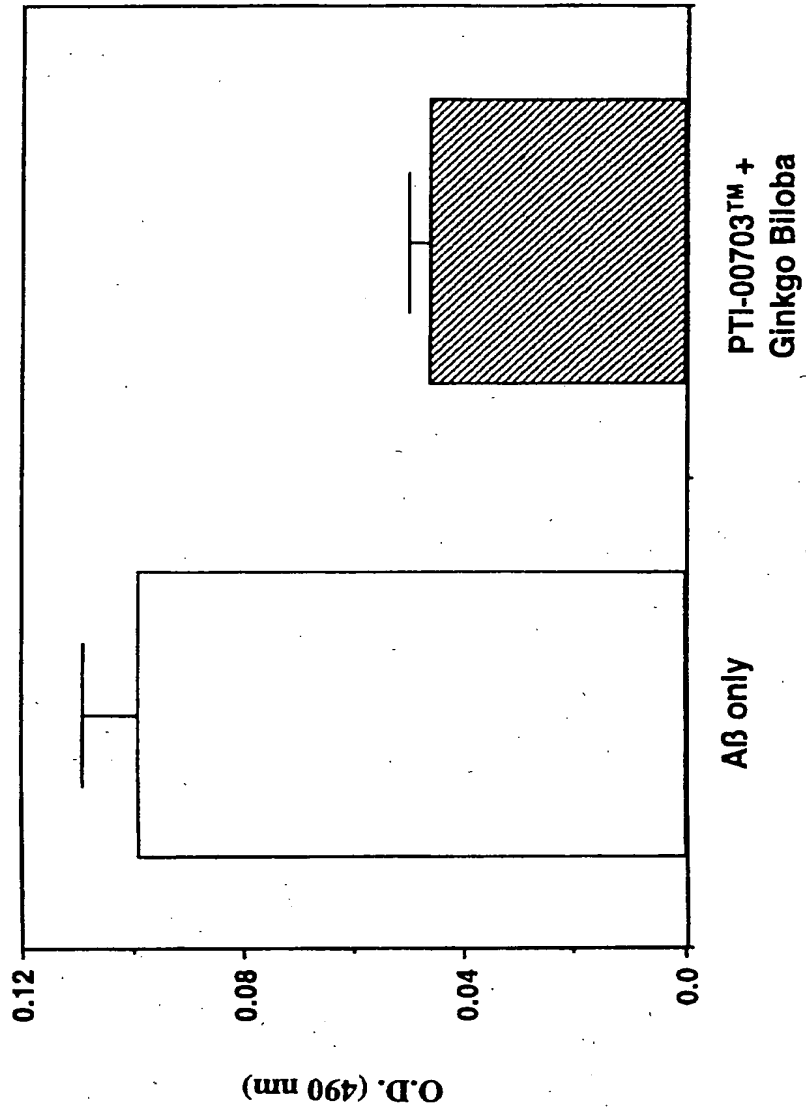
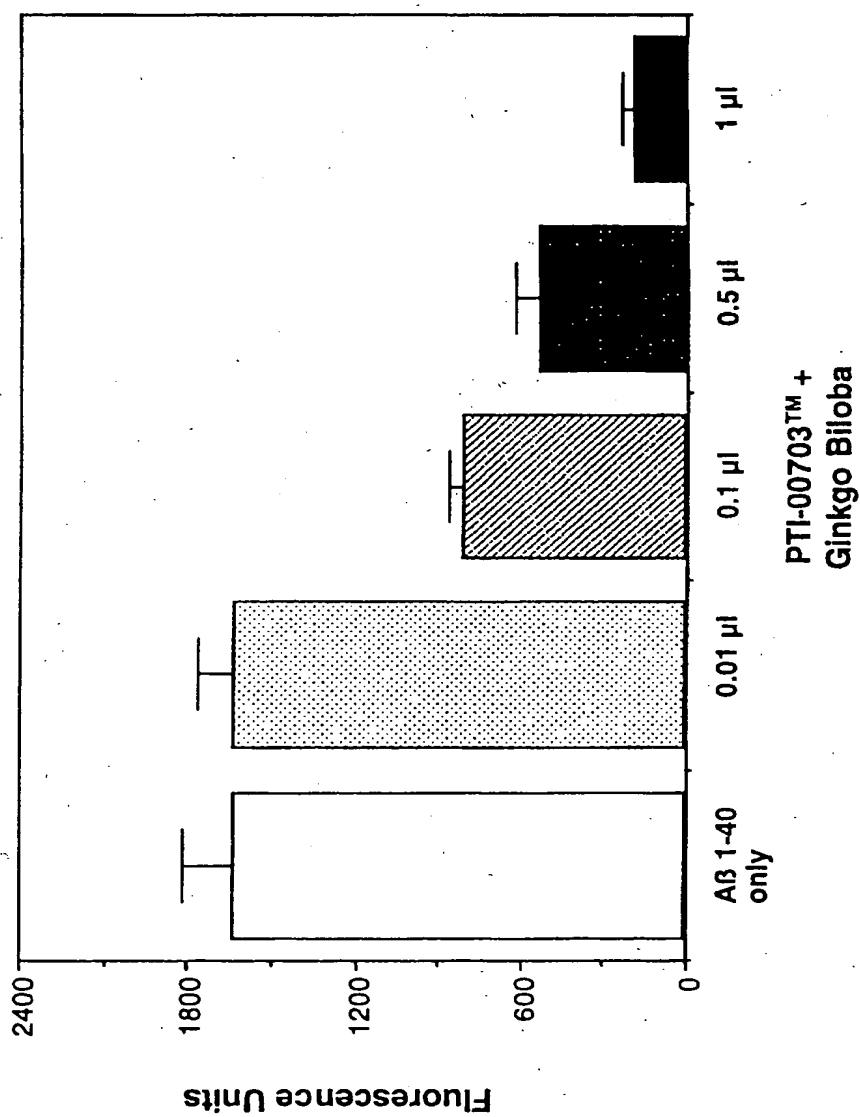


Fig. 3

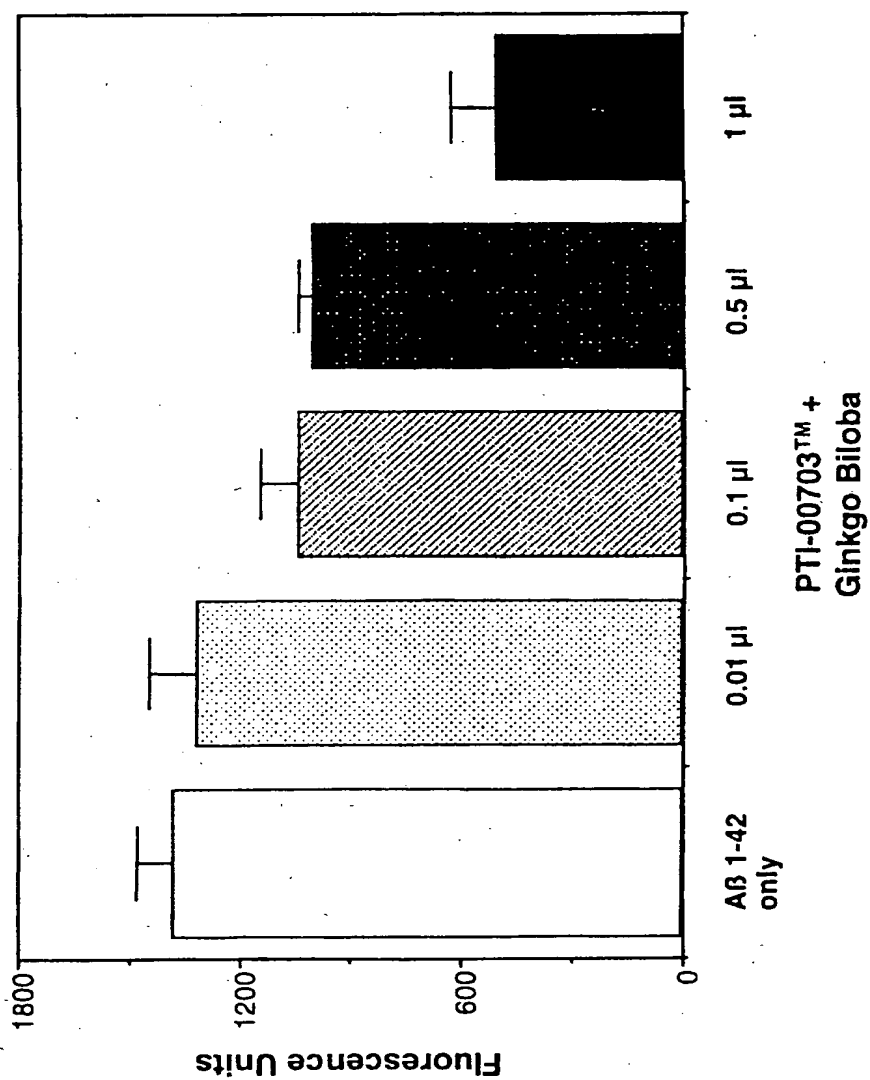
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Fig. 4



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Fig. 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19721

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 35/00

US CL :424/195.1, 464, 439

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1, 464, 439

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,571,441 A (ANDON et al.) 05 November 1996, see entire document.	1-16
Y	US 5,302,611 A (KEPLINGER et al.) 12 April 1994, see entire document, especially claim 19.	1-16
Y	Database BIOBUSINESS, Accession No. 97:61338, SCHECHTER, S, 'Cat's claw', abstract, Health Foods Business. July 1997, Vol. 43, No. 7, pages 77-78, abstract only.	1-16
A	US 5,681,569 A (KUZNICKI et al.) 28 October 1997, see entire document.	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
19 DECEMBER 1999

Date of mailing of the international search report
11 JAN 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/19721

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN, BIOSCIENCE

Uncaria tomentosa, Alzheimer's disease, amyloidoses, diabetes, ginkgo biloba, selenium, vitamins, folic acid, choline, echinacea, ginseng, niacin, nicotinate